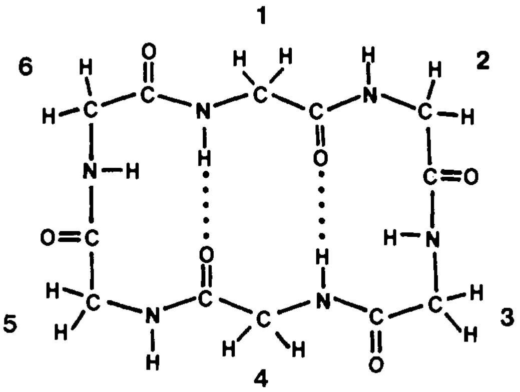
4730 *Biochemistry* **1981**, *20*, 4730–4738

Conformations of (X-L-Pro-Y)$_2$ Cyclic Hexapeptides. Preferred β-Turn Conformers and Implications for β Turns in Proteins[†]

Lila M. Gierasch,[‡] Charles M. Deber,[§] Vincent Madison,[⊥] Chien-Hua Niu,[‖] and Elkan R. Blout*

ABSTRACT: We have examined the effects of amino acid sequence on the formation of the β turn—a specific structural feature known to be of importance in proteins—through the use of a series of synthetic cyclic hexapeptides of sequence (X-L-Pro-Y)$_2$ (where X and Y are chosen from Gly, L- or D-Ala, L- or D-Phe, or L-Val in various combinations). Techniques used in establishing preferred β-turn conformers included proton and carbon-13 nuclear magnetic resonance and identification of general circular dichroism spectral types. A set of "rules" was formulated which correlate amino acid sequence with preferences for particular types of β turns in cyclic hexapeptides as follows: In a β turn consisting of four residues numbered sequentially *i* to *i* + 3, (1) the sequence -L-Pro-Gly- or -L-Pro-D-Y- leads to type II β turns (which have trans′ Pro residues in the *i* + 1 position of the $i + 3 \rightarrow i$ hydrogen-bonded turn), (2) the sequence -Gly-L-Pro-L-Y- (Y not L-Val) leads to type I β turns (which have cis′ Pro residues in the *i* + 1 position), and (3) the sequence -Gly-L-Pro-L-Val- or -D-X-L-Pro-L-Y- leads to type II′ β turns (which have cis′ Pro residues in the *i* + 2 position). Thus, among the peptides examined (none with all-L residues), Pro occurs in position *i* + 2 only when (a) D residues precede the L-Pro and L residues follow it *or* (b) L-Val follows L-Pro. In a concomitant aspect of this investigation, the occurrence in *cyclo*-(X-L-Pro-Y)$_2$ peptides of cis peptide bond containing conformers of two types, one C_2 symmetric (two cis) and the other asymmetric (one cis), was correlated with sequence and solvent. A survey of β turns in proteins reveals frequencies and positions of amino acid occurrence in β turns consistent with the hexapeptide-derived rules.

Cyclic peptides are excellent models for regions of a protein where the polypeptide chain reverses direction. Studies of several cyclic hexapeptides with repeating tripeptide sequences have revealed that a preferred conformation contains two $i + 3 \rightarrow i$ intramolecular hydrogen bonds such that the structure is essentially two linked β turns (Portnova et al., 1971; Schwyzer et al., 1972; Pease et al., 1973; Grathwohl et al., 1973; Torchia et al., 1972a,b; Kopple et al., 1969a,b, 1972, 1973, 1978), as shown in structure A.



A

The incorporation of proline residues into the sequences of these cyclic peptides is well justified by the high frequency of occurrence of Pro in β turns in proteins (Chou & Fasman, 1977; Smith & Pease, 1980) and in naturally occuring peptides (Ovchinnikov et al., 1970; Patel & Tonelli, 1973; Patel, 1973a,b; Urry & Walter, 1971; Karle et al., 1973, 1976; Karle, 1974; Reed & Johnson, 1973). The presence of proline residues in the hexapeptide sequence also reduces the number of possible β-turn conformers, since the Pro's can occupy only positions 2 and 5 or 3 and 6 in the hydrogen-bonded conformation shown in A (*i* + 1 or *i* + 2 positions in the turns). According to the nomenclature of Venkatachalam (1968) (using the numbering scheme of A), when the Pro's are in positions 2 and 5 (residues preceding Pro's are hydrogen bonded), then either type I or type II β turns may occur; when the Pro's are in positions 3 and 6 (residues following Pro's are hydrogen bonded), then type II′ β turns are present. However, proline does introduce an additional conformational variable, namely, the possibility of energetically competitive cis and trans isomers of the X-Pro peptide bond.

Our overall approach to peptide conformational analysis utilizes ^{1}H and ^{13}C NMR,[1] comparisons of experimental and theoretical circular dichroism spectra, and model building in a cooperative manner (Deber et al., 1976). In the present work, we have used these techniques to study in detail the role of sequence in determining preferred turns in peptides and proteins. For this purpose, we synthesized a series of cyclic hexapeptides of the type *cyclo*-(X-L-Pro-Y)$_2$, where X and Y are chosen from Gly, L- or D-Ala, L- or D-Phe, or L-Val, in

---

[†] From the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115. *Received September 25, 1980; revised manuscript received March 25, 1981.* This is paper 24 in a series entitled Cyclic Peptides. For the preceding paper, see Easwaran et al. (1979). This work was supported, in part, by U.S. Public Health Service Grants AM07300 and AM10794 (E.R.B.), GM27616 (L.M.G.), and RR00292 (Carnegie-Mellon NMR Facility). Acknowledgment is also made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research (L.M.G.). Portions of this work are derived from the dissertation of Lila M. Gierasch (Pease) presented in partial fulfillment of the requirements for a Ph.D. degree, Harvard University, 1975.

[‡] Present address: Department of Chemistry, University of Delaware, Newark, DE 19711.

[§] Present address: Department of Biochemistry, University of Toronto, and Research Institute, Hospital for Sick Children, Toronto, Ontario, M5G 1X8 Canada.

[⊥] Present address: Department of Medicinal Chemistry, University of Illinois Medical Center, Chicago, IL 60680.

[‖] Present address: National Institutes of Health, the Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, MD 20205.

[1] Abbreviations used: NMR, nuclear magnetic resonance; CD, circular dichroism; *t*-Boc, *tert*-butyloxycarbonyl; OBz, benzyl ester; TLC, thin layer chromatography; IR, infrared; TFE, trifluoroethanol.

various combinations. Some cyclic hexapeptides of this type have been examined in previous NMR studies (Schwyzer et al., 1972; Pease et al., 1973; Grathwohl et al., 1973; Torchia et al., 1972a,b; Kopple et al., 1973, 1974). A general preference was exhibited for a β-turn conformation in which the residues preceding the Pro's were intramolecularly hydrogen bonded, rather than the alternative with the residues following the Pro's hydrogen bonded. The occurrence of sequence-dependent asymmetric one-cis (Torchia et al., 1972a) or C_2-symmetric two-cis (Torchia et al., 1972b; Kopple et al., 1973) structures has also been demonstrated. By combining knowledge obtained from conformational studies on the new series of cyclic hexapeptides reported herein with existing data, we have been able to examine structure/sequence relationships in nearly all essential variations. Finally, a set of "rules" for prediction of β-turn types as a function of peptide sequence has been formulated.

Experimental Procedures

Synthesis. Nine cyclic hexapeptides of repeating tripeptide sequences, *cyclo*-(X-Pro-Y)$_2$, were synthesized by cyclodimerization of tripeptide active esters. Amino acid starting materials (Fox Chemical Co., Los Angeles, CA, or Schwartz BioResearch, Inc., Orangeburg, NY) were protected by *tert*-butyloxycarbonyl groups on N termini or benzyl esters on C termini. Synthetic procedures followed methods which have been described in detail [see, for example, Torchia et al. (1972a,b) and Deber & Blout (1974)]. Couplings were accomplished by mixed anhydride reactions using *N*-methylmorpholine and isobutyl chloroformate. Deprotection was effected either by (1) treatment with HCl–ether or HCl–ethyl acetate for *t*-Boc groups or (2) hydrogenation in *tert*-butyl alcohol in the presence of 10% palladium/charcoal for OBz esters. Either *p*-nitrophenyl or pentachlorophenyl esters were cyclized; the appropriate precursor peptides were activated by using dicyclohexylcarbodiimide as a coupling agent. Solvents used were of analytical grade, with the exception of pyridine which was spectrophotometric grade. All were used without further purification. Precursors were crystallized, if possible. *All* N-protected tripeptide active esters were crystallized, but the corresponding hydrochloride salts were not, as it was desirable to use these compounds without delay. Thin layer chromatography on Quanta/Gram O1F precoated plates (Quanta Industries, Fairfield, NJ) was employed to monitor workups and product purity. Spots were visualized with a chlorine/tolidine spray. Infrared spectroscopy, melting points, and ^{1}H NMR were used to check the identity of intermediate products.

In the cyclodimerizations tripeptide active ester hydrochlorides were dissolved in a small volume of dimethylformamide (dried over Na_2SO_4 and neutralized with acetic acid) and added dropwise over a 2–3-h period to a large volume of stirred pyridine (ca. 500 mL for 2 mmol of active ester). Reaction was allowed to proceed at room temperature for approximately 3 days, at which point the solution was bright yellow. Workup was achieved by evaporation of solvents and trituration of the oily yellow residue with ether followed by treatment of the remaining material with Rexyn I-300 mixed-bed ion-exchange resin (ca. 30 g, Fisher Scientific Co., Fair Lawn, NJ) in 50–100 mL of ethanol–water (50:50 v/v) for 2 h. At this point the crude products after evaporation of solvents were solid and showed predominantly one major component on TLC in all cases. Crystallization then yielded the desired product in a homogeneous form. Cyclic peptide products were characterized by elemental analysis (Spang Microanalytical Laboratory, Ann Arbor, MI), melting point, IR spectra, high-resolution mass spectrometry (performed at the Massachusetts Institute of Technology Regional Facility), and NMR spectra. Further details regarding specific protocols and characterizations of specific peptides have been reported elsewhere (Pease, 1975).

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were run on Varian T-60, HA-100, XL-100, HR-220, or HR-300 instruments or on the 250-MHz instrument at Carnegie-Mellon Institute. Fourier transform methods were employed on the HA-100 and XL-100 when nonprotonated solvents were used. The temperature was calibrated with an ethylene glycol sample for temperatures above 20 °C, using the correlations given by Varian. Proton–proton spin decoupling experiments were done either on the Carnegie-Mellon instrument or on the XL-100 (in Fourier transform mode).

^{13}C NMR spectra of the peptides in deuterated solvents were obtained on a Varian CFT-20 instrument operating at 20 MHz. Spectra were run proton decoupled, in Fourier transform mode, with a 60° pulse and 0.5-s acquisition time. An internal deuterium lock was used. Chemical shifts are given in parts per million downfield from internal Me_4Si.

All NMR samples were 0.02–0.05 M. Deuterated solvents were purchased from Merck Sharp & Dohme or from Thompson Packard. Solutions were filtered through glass wool to remove any particulate matter.

CD Spectroscopy. CD spectra of ca. 2×10^{-4} M peptide solutions in water or trifluoroethanol were recorded on a Cary 60 spectropolarimeter with 6001 attachment. CD extrema are expressed as mean residue ellipticities ($m_\theta = 100\,\theta/nCl$, where θ is the measured ellipticity in degrees, l is the path length in centimeters, C is the molar concentration, and n is the number of residues).

CD Calculations. Since glycine has more conformational freedom than any other amino acid, the stable conformational regions for all *cyclo*-(X-Pro-Y)$_2$ peptides will be contained within the regions found for *cyclo*-(Gly-Pro-Gly)$_2$. Local intramolecular potential energy minima for C_2-symmetric conformers of *cyclo*-(Gly-Pro-Gly)$_2$ have been reported (Deber et al., 1976). For conformers in the regions of these minima, CD spectra for the cyclic hexapeptides were computed using reported methods (Madison, 1973; Madison et al., 1974).

Results

Conformational Types as a Function of Primary Sequence.[2] Both ^{1}H and ^{13}C NMR spectra reveal the presence of populations of multiple slowly interconverting conformers in several of the cyclic hexapeptides. On the basis of the pattern and number of resonances in the diagnostic Pro C^β and C^γ region of the ^{13}C spectra, it is possible to characterize the conformers as all-trans (C_2 symmetric), two-cis (containing two cis X–Pro peptide bonds but also C_2 symmetric), or one-cis (asymmetric), and to estimate the fractions of each (see Figure 1 and Table I).

On the basis of the occurrence of cis conformers, the cyclic hexapeptides fall into three classes: (1) *cyclo*-(L-X-L-Pro-D-Y)$_2$ and *cyclo*-(L-X-L-Pro-Gly)$_2$, which, like the previously described peptides *cyclo*-(L-Ser-L-Pro-Gly)$_2$ (Torchia et al., 1972b) and *cyclo*-(L-X-L-Pro-D-Phe)$_2$ where X = L-Ala, L-His, or L-Orn (Kopple et al., 1974), adopt in addition to the all-trans conformation a C_2-symmetric two-cis form; (2) *cyclo*-(Gly-L-Pro-L-Y)$_2$, which, like *cyclo*-(Gly-L-Pro-L-Ser)$_2$

---

[2] For an explanation of conventions used in dihedral angle nomenclature, see IUPAC–IUB Commission on Biochemical Nomenclature (1970).

Table I: Distribution of All-Trans and One- or Two-Cis Conformers for *cyclo*-(X-L-Pro-Y)$_2$ Peptides[a]

peptide	solvent	all-trans (%)	one-cis (%)	two-cis (%)
cyclo-(L-Ala-L-Pro-Gly)$_2$	D_2O	88	0	12
cyclo-(L-Ser-L-Pro-Gly)$_2$[b]	D_2O	75	0	25
	$(CD_3)_2SO$	20	0	80
cyclo-(L-Val-L-Pro-Gly)$_2$	TFE-d_3	58	0	42
	D_2O	13	0	87
	$(CD_3)_2SO$	<20	0	>80
cyclo-(L-Phe-L-Pro-Gly)$_2$	TFE-d_3	36	0	64
	$CDCl_3$	16	0	84
	$(CD_3)_2SO$	18	0	82
cyclo-(L-Ala-L-Pro-D-Phe)$_2$	$(CD_3)_2SO$	53	0	47
cyclo-(L-Orn-L-Pro-D-Phe)$_2$	D_2O	42	0	58
cyclo-(L-His-L-Pro-D-Phe)$_2$	D_2O	21	0	79
cyclo-(Gly-L-Pro-L-Ala)$_2$	D_2O	76	24	0
cyclo-(Gly-L-Pro-L-Ser)$_2$	D_2O	77	23	0
cyclo-(Gly-L-Pro-L-Phe)$_2$	$(CD_3)_2SO$	100	0	0
cyclo-(Gly-L-Pro-L-Tyr)$_2$	D_2O	75	25	0
cyclo-(Gly-L-Pro-L-Val)$_2$	TFE-d_3	73	27	0
	$CDCl_3$	68	32	0
	D_2O	58	42	0
	$(CD_3)_2SO$	23	77	0

[a] Measured by ^{13}C NMR; uncertainty ±5%. [b] Measured by ^1H NMR (Torchia et al., 1972b).

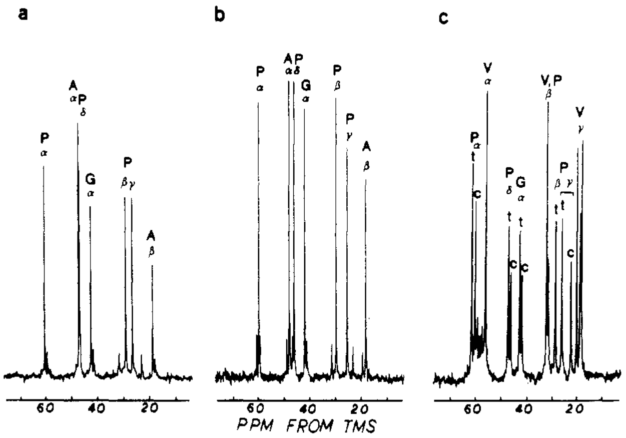


FIGURE 1: ^{13}C NMR spectra (20 MHz) illustrating mixtures of all-trans and either two-cis or one-cis structures, 30 °C: (a) *cyclo*-(L-Ala-L-Pro-Gly)$_2$ in D_2O, 50 mg/mL; (b) *cyclo*-(Gly-L-Pro-Ala)$_2$ in D_2O, 50 mg/mL; (c) *cyclo*-(Val-L-Pro-Gly)$_2$ in TFE-d_3. In (b), note the presence of two signals for the minor (one-cis) species for some carbons (Pro C^α, Ala C^α, for example), indicating asymmetry. In (c), resonances are labeled "t" for all-trans or "c" for two-cis. The estimated ratios of all-trans to two- or one-cis conformers in these examples are (a) 88:12 (all-trans:two-cis), (b) 76:24 (all-trans:one-cis), (c) 58:42 (all-trans:two-cis). In (c), the small broad signal at ca. 63 ppm is due to solvent.

(Torchia et al., 1972a), adopt to varying extents a one-cis, asymmetric conformation as well as the all-trans form, and (3) peptides with X and Y = Gly or D residue, which take up only the all-trans form.

Accessibility and Hydrogen Bonding of Peptide N–H's. From ^1H NMR data on the peptide N–H resonances (Table II), a general preference for a β conformer of the type illustrated in structure A is manifest by all-trans conformers of the cyclic hexapeptides. The evidence for such a conformation is a demonstrated solvent shielding of one pair of N–H's relative to the other [signaled by (1) a reduced temperature dependence relative to exposed N–H's in solvents which are good hydrogen bond acceptors and (2) a chemical shift which is relatively independent of solvent]. In all cases except *cyclo*-(Gly-L-Pro-L-Val)$_2$ and *cyclo*-(D-Phe-L-Pro-L-Val)$_2$, the N–H's of the X residues (preceding the Pro's) are solvent shielded relative to the N–H's of the Y residues. This result is consistent with a β conformation with the X residues extended and Pro's in positions 2 and 5. By contrast, *cyclo*-(Gly-L-Pro-L-Val)$_2$ and *cyclo*-(D-Phe-L-Pro-L-Val)$_2$ both show

Table II: ^1H NMR Data on Peptide N–H Resonances[a]

		X			Y		
peptide	solvent	δ	$J_{N\alpha}$ (Hz)	$\Delta\delta/\Delta T^b$	δ	$J_{N\alpha}$ (Hz)	$\Delta\delta/\Delta T^b$
cyclo-(Gly-L-Pro-Gly)$_2$	$(CD_3)_2SO$	7.57	4.0, 3.0	1.3	8.62	5.7, 5.5	5.3
	H_2O	7.98			8.74		
cyclo-(D-Phe-L-Pro-Gly)$_2$	$(CD_3)_2SO$	7.89	8.0	1.7	8.26	6.0, 6.5	4.8
	$CDCl_3$	7.76	7.5		7.71		
cyclo-(Gly-L-Pro-D-Phe)$_2$	$(CD_3)_2SO$	7.69	$\Sigma = 7.0$	3.2	8.80	8.0	7.0
	CD_2Cl_2	7.45			6.80		
cyclo-(D-Ala-L-Pro-D-Ala)$_2$[c]	$(CD_3)_2SO$	7.66	8.0	1.6	8.58	7.5	6.4
	H_2O	7.87	8.0		8.65	6.5	
	$CDCl_3$	7.60			6.65		
cyclo-(L-Ala-L-Pro-Gly)$_2$	$(CD_3)_2SO$	7.90	6.5	0.82	8.55	$\Sigma = 9$	6.2
	H_2O	8.23	6.5		8.70	$\Sigma = 11.5$	
cyclo-(L-Phe-L-Pro-Gly)$_2$ (cis)	$(CD_3)_2SO$	8.36	~6	6.2	8.06	$\Sigma = 14$	5.4
	$CDCl_3$	6.82	~6		~7		
cyclo-(L-Val-L-Pro-Gly)$_2$ (cis)	$(CD_3)_2SO$	8.05	~6	6.6	8.00	$\Sigma = 14$	5.8
	H_2O	8.03	~6		8.38	$\Sigma = 14$	
(trans)	TFE		~8			$\Sigma = 14$	
cyclo-(Gly-L-Pro-L-Ala)$_2$	$(CD_3)_2SO$	7.45	$\Sigma = 9.5$	0.1	8.28	8.3	7.5
	H_2O	7.82	$\Sigma = {\sim}8$		8.35	~8	
	$CDCl_3$	7.81	$\Sigma = 7.5$		6.92	8.5	
cyclo-(Gly-L-Pro-L-Phe)$_2$	$(CD_3)_2SO$	7.52	$\Sigma = {\sim}9$	0.69	8.17	8.5	6.4
	$CDCl_3$	7.78	$\Sigma = {\sim}9$		7.3		
cyclo-(Gly-L-Pro-L-Val)$_2$ (trans)	H_2O	8.20	$\Sigma = {\sim}12$		7.85	~8	
(trans)	$CDCl_3$	7.31	$\Sigma = {\sim}12$	4.6	7.09	~9	2.0
cyclo-(D-Phe-L-Pro-L-Val)$_2$	$(CD_3)_2SO$	8.43	~5	5.9	6.82	~8	1.6
	$CDCl_3$	7.60	~6		6.94	9.5	

[a] Data are given for 0.02 M solutions, 30 °C. An internal reference of tetramethylsilane was used in all nonaqueous solutions; on the HA-100, Me_4Si was also used as an internal lock. Chemical shifts in H_2O are referenced to the water proton resonance, assumed to be 4.80 ppm (δ scale); in D_2O to the HDO signal, assumed to be 4.61 ppm. Variable-temperature experiments were accomplished by the use of the Varian variable-temperature accessory, using dry N_2 as a heat-exchanging gas. [b] $\times 10^3$ ppm/deg, over the temperature range 30–80 °C. [c] The two alanine residues in this compound were distinguished through the use of specifically deuterated analogues (C.-H. Niu, unpublished results).

Table III: ^{13}C Proline C^{β} and C^{γ} Chemical Shifts of All-Trans Conformers[a]

peptide	δ (C^{β})	δ (C^{γ})	$\Delta\delta_{\beta\gamma}$
cyclo-(D-Phe-L-Pro-L-Val)$_2$[b]	30.9	25.1	5.8
cyclo-(D-Phe-L-Pro-L-Ala)$_2$[b]	30.7	25.1	5.6
cyclo-(Gly-L-Pro-L-Val)$_2$	30.5	25.4	5.1
cyclo-(Gly-L-Pro-L-Ser)$_2$	30.4	25.5	4.9
cyclo-(Gly-L-Pro-L-Ala)$_2$	30.1	25.5	4.6
cyclo-(Gly-L-Pro-L-Tyr)$_2$	29.9	25.4	4.5
cyclo-(D-Ala-L-Pro-Gly)$_2$	29.9	25.6	4.3
cyclo-(Gly-L-Pro-L-Phe)$_2$[b]	30.0	25.8	4.2
cyclo-(D-Phe-L-Pro-Gly)$_2$[b]	29.9	25.7	4.2
cyclo-(Gly-L-Pro-Gly)$_2$	29.8	25.7	4.1
cyclo-(D-Ala-L–Pro-D-Ala)$_2$	29.7	25.7	4.0
cyclo-(Gly-L-Pro-D-Ala)$_2$	29.3	25.9	3.4
cyclo-(Gly-L-Pro-D-Phe)$_2$[b]	29.3	26.2	3.1
cyclo-(L-His-L-Pro-D-Phe)$_2$	29.4	26.4	3.0
cyclo-(L-Phe-L-Pro-Gly)$_2$[c]	28.5	25.6	2.9
cyclo-(L-Val-L-Pro-Gly)$_2$[c]	28.5	25.7	2.8
cyclo-(L-Val-L-Pro-Gly)$_2$	29.3	26.5 or 25.9	2.8 or 3.4
cyclo-(L-Ala-L-Pro-Gly)$_2$	29.4	26.6	2.8
cyclo-(L-Orn-L-Pro-D-Phe)$_2$	29.0 or 28.2	26.3	2.7 or 1.9
cyclo-(L-Phe-L-Pro-Gly)$_2$[b]	29.5	27.0	2.5
cyclo-(L-Val-L-Pro-Gly)$_2$[b]	29.5	27.0	2.5
cyclo-(L-Ala-L-Pro-D-Ala)$_2$	29.0	26.7	2.3
cyclo-(L-Phe-L-Pro-Gly)$_2$[d]	29.2	27.1	2.1
cyclo-(L-Ala-L-Pro-D-Phe)$_2$[b]	28.7	27.0	1.7

[a] Chemical shifts are given downfield from Me_4Si (defined as 193.7 ppm from external $^{13}CS_2$). Data are for D_2O solutions unless otherwise indicated. [b] In $(CD_3)_2SO$. [c] In TFE-d_3. [d] In $CDCl_3$.

solvent shielding of the Val N–H's relative to those of the residue preceding the Pro's, although the data for the former peptide are less definitive than those for the other peptides. In these cases, the alternative β structure is indicated with the Pro's in positions 3 and 6, and the Y residues extended.

The two peptides which were shown by ^{13}C NMR to take up predominantly C_2-symmetric two-cis conformations, viz., *cyclo*-(L-Val-L-Pro-Gly)$_2$ and *cyclo*-(L-Phe-L-Pro-Gly)$_2$, exhibit the same degree of solvent exposure for each of their N–H resonances (X and Y) in the two-cis conformer.

Trends in Proline ^{13}C NMR Chemical Shifts among β Conformers. Earlier work (Madison et al., 1974; Siemion et al., 1975; Deber et al., 1976; Pease & Watson, 1978; Pease et al., 1979) has established that variations in chemical shifts of the Pro C^{β} and C^{γ} resonances *within* the region associated with a trans X–Pro bond geometry can be correlated with certain values of the Pro ψ angle. Thus, when eclipsing of the Pro $C^{\beta}H_2$ and C=O occurs near $\psi = 60°$,[2] then the C^{β} resonance will be observed at higher field than in the absence of such eclipsing. Furthermore, a proposed correlation between proline ψ angle and the chemical shift difference between the resonances of C^{β} and C^{γ} (Siemion et al., 1975) has been shown to be entirely consistent with conformers deduced from CD and ^{1}H nuclear Overhauser effect data (Madison & Kopple, 1980).

Data from the nine synthesized cyclic hexapeptides and from a number of others whose spectra were recorded for purposes of comparison reveal a distinct trend of the $\Delta\delta_{\beta\gamma}$, which is largest for the peptides of sequence *cyclo*-(D-X-L-Pro-L-Y)$_2$ and smallest for those of sequence *cyclo*-(L-X-L-Pro-D-Y)$_2$ (see Table III). For comparison, the resonances of the Pro C^{β} and C^{γ} of gramicidin S (which contains type II' β turns with D-Phe-L-Pro sequences in the turns) occur (in dimethyl sulfoxide solution) at 30.3 and 24.2 ppm, respectively ($\Delta\delta_{\beta\gamma} = 6.1$ ppm) (Sogn et al., 1974).

Comparison of Experimental and Calculated CD Spectra. CD spectra of four general types are observed for various *cyclo*-(X-L-Pro-Y)$_2$ peptides (Figure 2). Three of these types are attributable predominantly to all-trans β conformers and one [that of *cyclo*-(L-Val-L-Pro-Gly)$_2$ in H_2O] predominantly to a two-cis species, based on the analysis of ^{13}C NMR spectra (vide supra and Table I). Spectra of *cyclo*-(Gly-L-Pro-Gly)$_2$, *cyclo*-(L-Ala-L-Pro-Gly)$_2$, *cyclo*-(Gly-L-Pro-D-Ala)$_2$, and *cyclo*-(L-Ala-L-Pro-D-Ala)$_2$ in both H_2O and TFE and *cyclo*-(L-Val-L-Pro-Gly)$_2$ in TFE all resemble one another and are characterized by a small negative band at ca. 225 nm and a larger positive band at ca. 200 nm (see Figure 2a and Table IV). Some differences may be noted in the ellipticities of the bands and in the occurrence in some cases of a significant negative ellipticity at short wavelengths. These spectra are termed type B, by comparison with Woody's (1974) classification of calculated spectra of β turns.

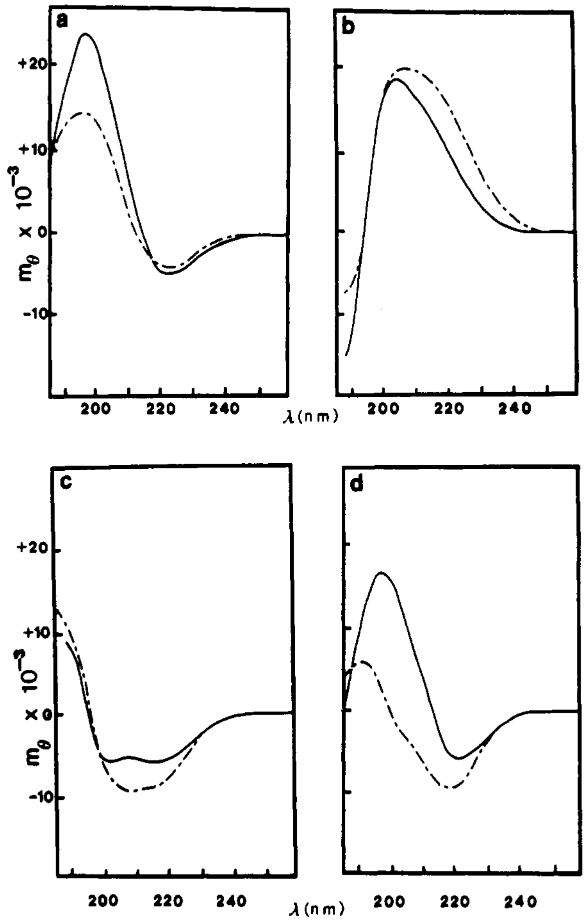


FIGURE 2: Representative circular dichroism spectra of (X-L-Pro-Y)$_2$ cyclic hexapeptides, 2×10^{-4} M, 20 °C, in H_2O (---) and TFE (—): (a) *cyclo*-(L-Ala-L-Pro-Gly)$_2$, type B; (b) *cyclo*-(D-Ala-L-Pro-D-Ala)$_2$, type C; (c) *cyclo*-(Gly-L-Pro-L-Ala)$_2$, type C; (d) *cyclo*-(L-Val-L-Pro-Gly)$_2$, largely two-cis (H_2O), 58:42, all-trans:two-cis (TFE).

cyclo-(D-Ala-L-Pro-D-Ala)$_2$ and *cyclo*-(D-Ala-L-Pro-Gly)$_2$ have broad spectra lacking a long-wavelength minimum and are termed type C′ after Woody (1974) (Figure 2b and Table IV).

Spectra of *cyclo*-(Gly-L-Pro-L-Ala)$_2$, *cyclo*-(Gly-L-Pro-L-Val)$_2$, and *cyclo*-(D-Phe-L-Pro-L-Val)$_2$ are characterized by double-minima at ca. 200 and 225 nm and positive ellipticity at shorter wavelengths (Figure 2c and Table IV). These spectra are similar in shape to those reported for gramicidin S (Urry et al., 1968) and for the tyrocidines (Laiken et al., 1969) but with smaller ellipticities. According to Woody's (1974) classification, these are type C spectra.

The observed type B (Figure 2a) and type C′ (Figure 2b) CD spectra are well matched by those predicted for low-energy type β-turn conformers of *cyclo*-(Gly-L-Pro-Gly)$_2$. Type C′

Table IV: CD Extrema of (X-L-Pro-Y)$_2$ Cyclic Hexapeptides[a]

peptide	solvent	CD type	CD extrema					
			m_θ ($\times 10^{-3}$)	λ (nm)	m_θ ($\times 10^{-3}$)	λ (nm)	m_θ ($\times 10^{-3}$)	λ (nm)
cyclo-(Gly-L-Pro-Gly)$_2$	H_2O	B	−4.0	222	9.0	198	2.0	190
	TFE	B	−3.0	225	15.8	199	4.0	190
cyclo-(Gly-L-Pro-D-Ala)$_2$	H_2O	B	−2.0	230	26.0	202	−8.0	185
	TFE	B	−4.4	226	27.0	200	−7.0	188
cyclo-(L-Ala-L-Pro-Gly)$_2$	H_2O	B	−4.6	222	15.4	195		
	TFE	B	−5.2	223	24.4	196	13.0	188
cyclo-(D-Ala-L-Pro-D-Ala)$_2$	H_2O	C′	19.0[b]	215	20.0	205	−7.0	190
	TFE	C′	14.0[b]	214	19.0	203	−10.0	190
cyclo-(L-Val-L-Pro-Gly)$_2$	H_2O		−10.0	218	6.4	191		
	TFE	B	−6.0	222	17.0	196	3.0	188
cyclo-(Gly-L-Pro-L-Ala)$_2$	H_2O	C	−9.2	215	−9.6	208	12.0	188
	TFE	C	−6.0	218	−6.0	202	8.0	190
cyclo-(Gly-L-Pro-L-Val)$_2$	H_2O	C	−6.0[b]	220	−15.0	202	6.0	188
	TFE	C	−9.0[b]	222	−15.0	201	3.0	190
cyclo-(D-Ala-L-Pro-Gly)$_2$	H_2O	C′	10.0	215			−14.0	187
cyclo-(L-Ala-L-Pro-D-Ala)$_2$	H_2O	B	−4.0	227	31.0	201	7.0	185
cyclo-(D-Phe-L-Pro-L-Val)$_2$	CH_3CN	C	−8.7	223	−16.3	201		

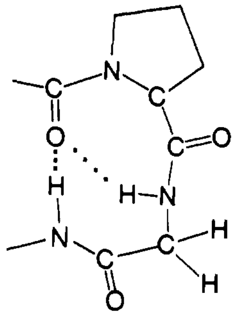
[a] c 2 × 10^{-4} M, 20 °C. [b] Shoulder.

Table V: Summary of Results on (X-L-Pro-Y)$_2$ Cyclic Hexapeptides[a]

X-L-Pro-Y sequence	pref β turn	chem shift int$_{N-H}$	$\Delta\delta/\Delta T$[c]		$\Delta\delta_{\beta\gamma}$[d]	CD type	γ turn?	Pro ψ	two-cis	one-cis
			X	*Y*						
L-X-L-Pro-D-Y	II	8.0–8.2			3.0–1.7	B	yes	trans′	yes	no
L-X-L-Pro-Gly	II	7.9–8.0	0.8	6.2	2.9–2.1	B	yes	trans′	yes	no
Gly-L-Pro-D-Y	II	7.7	3.2	7.0	3.4–3.1	B	yes	trans′	no	no
Gly-L-Pro-Gly	II	7.6	1.3	5.3	4.1	B	yes	trans′	no	no
D-X-L-Pro-D-Y	II	7.7	1.6	6.4	4.0	C′	no	trans′	no	no
D-X-L-Pro-Gly	II	7.9	1.7	4.8	4.3–4.2	C′	no	trans′	no	no
Gly-L-Pro-L-Y	I	7.4–7.8	0.1–0.7	6.4–7.5	4.9–4.5	C	no	cis′	no	yes
Gly-L-Pro-L-Val	II′	7.1[e]	4.6[e]	2.0[e]	5.1	C	no	cis′	no	yes
D-Phe-L-Pro-L-Val	II′	7.0	5.9	1.6	5.8–5.6	C	no	cis′	no	no

[a] Where a range of values is given, several compounds are included. [b] In $(CD_3)_2SO$ unless otherwise indicated. [c] In $(CD_3)_2SO$; $\times 10^3$ ppm. [d] Data in D_2O usually; see Table III. [e] In $CDCl_3$.

spectra are predicted for conformers with larger proline ψ angles (>ca. 100°) and type B spectra for smaller angles (70–90°). Inspection of these latter conformers reveals that they contain significant contributions from an $i + 2 \rightarrow i$ (γ turn) type hydrogen-bonding interaction in addition to the β-turn hydrogen-bonding array (see structure B).



B

In addition to those type B and C′ spectra found in low-energy regions for type II β-turn conformers, there were also many examples of these spectral types in other low-energy regions for *cyclo*-(Gly-L-Pro-Gly)$_2$ (this work) and for linear tetrapeptides containing β turns (Woody, 1974). Although these other examples are characterized by the same general shape and extrema, both the predicted $\pi \rightarrow \pi^*$ band (near 210 nm) and the negative band near 190 nm are blue shifted about 10 nm from the values for the type II β-turn region. The best fit with experimental spectra is with the spectra predicted for type II β turns.

Type C spectra (Figure 2c) are predicted only rarely, in most cases for conformers containing cis′ proline ψ angles. Furthermore, all conformers with cis′ proline ψ angles have a long wavelength (220–230 nm) minimum in their predicted CD spectra. Therefore, the predicted CD spectra are consistent with our inferences of type I and type II′ β conformers (which have cis′ Pro ψ) for peptides with type C CD spectra. Note that this correlation is more general than that proposed by Bush et al. (1978) wherein type C spectra were associated only with type II′ turns. CD spectra (Figure 2d) observed for the two-cis conformer are well matched by those predicted for conformers similar to that postulated (Table VI).

Conformational Interpretation. The (L-X-Pro-Gly)$_2$ cyclic hexapeptides are characterized by several distinctive experimental parameters: (1) a small value of $\Delta\delta_{\beta\gamma}$; (2) solvent inaccessibility of the X-residue N–H's and relatively low-field chemical shifts for the NMR resonances of these N–H's, (3) type B CD spectra, and (4) a solvent- and sequence-dependent conformational equilibrium between all-trans and two-cis structures. (For purposes of comparison among the *cyclo*-(X-L-Pro-Y)$_2$ peptides, these properties are listed in Table V.) These observations are all consistent with the occurrence of an all-trans β conformation containing type II β turns in which the proline ψ angles are low trans′ (ψ = 70–80°) and there are γ turns ($i + 2 \rightarrow i$ hydrogen bonds) between the X C=O's and the Y N—H's.

A CPK model of the proposed β conformation is shown in Figure 3a. Note the presence of simultaneous $i + 2 \rightarrow i$ and

Table VI: Dihedral Angles of Proposed Solution Conformations of (X-L-Pro-Y)$_2$ Cyclic Hexapeptides[a]

	X	L-Pro	Y
cyclo-(L-X-L-Pro-Gly)$_2$ β Conformer with Type II β Turns and with γ Turns			
φ	−160	−70	−170
ψ	170	80	60
ω	180	180	180
Two-Cis Structure			
φ	−170	−70	160
ψ	100	170	−160
ω	0	180	180
cyclo-(Gly-L-Pro-D-Y)$_2$ and *cyclo*-(Gly-L-Pro-Gly)$_2$ β Conformer with Type II β Turns and Slight γ-Turn Contribution			
φ	170	−70	150
ψ	180	90	0
ω	180	180	180
cyclo-(D-X-L-Pro-D-Y)$_2$ and *cyclo*-(D-X-L-Pro-Gly)$_2$ β Conformer with Type II β Turns (No γ Turns)			
φ	150	−60	120
ψ	170	150	0
ω	180	180	180
cyclo-(Gly-L-Pro-L-Y)$_2$ (Y not L-Val) β Conformer with Type I β Turns			
φ	150	−40	−150
ψ	−170	−50	100
ω	180	180	180
cyclo-(Gly-L-Pro-L-Val)$_2$ and *cyclo*-(D-Phe-L-Pro-L-Val)$_2$ β Conformer with Type II′ β Turns			
φ	70	−80	−150
ψ	−150	−50	160
ω	180	180	180

[a] In degrees at ±15 °C.

$i + 3 \rightarrow i$ hydrogen bonding to the X C═O's. Approximate dihedral angles for this all-trans conformation (see Table VI) were obtained through inspection of a molecular model, fitting of dihedral angles and coupling constants (Bystrov et al., 1973; Ramachandran et al., 1971; DeMarco ett al., 1978), and comparison of CD extrema with those calculated for related conformers. Some degree of conformational mobility and consequent averaging of experimental parameters cannot be completely ruled out, particularly in the local region of the glycines, but the consistent fit of all spectral data by this general conformation strongly supports the conclusion that it is the preferred β conformation. This conformation is very similar to that proposed by Kopple et al. (1974) for the *cyclo*-(L-X-L-Pro-D-Y)$_2$ peptides, although for the latter compounds no γ-turn contribution was proposed. On the basis of the ^{13}C data, we suggest that the *cyclo*-(L-X-L-Pro-D-Y)$_2$ peptides also adopt conformations in solution with both $i + 2 \rightarrow i$ and $i + 3 \rightarrow i$ hydrogen-bonding contributions.

A unique conformation can be ascribed to the two-cis structure observed to be in slow equilibrium with the all-trans in the *cyclo*-(L-X-L-Pro-Gly)$_2$ [and *cyclo*-(L-X-L-Pro-D-Y)$_2$ (Kopple et al., 1974)] peptides. This conformation (dihedral angles given in Table VI) is consistent with all spectral data and has a calculated CD which matches well the experimental spectrum. Relief of steric hindrance between an L-X side chain and the Pro δ-methylene group is the apparent driving force favoring the two-cis structure. The data for distributions between all-trans and two-cis structures (Table I) suggest further that the presence of D residues following the L-Pro's leads to more two-cis and that solvents of high polarity and good hydrogen-bond accepting capability also favor the two-cis species.

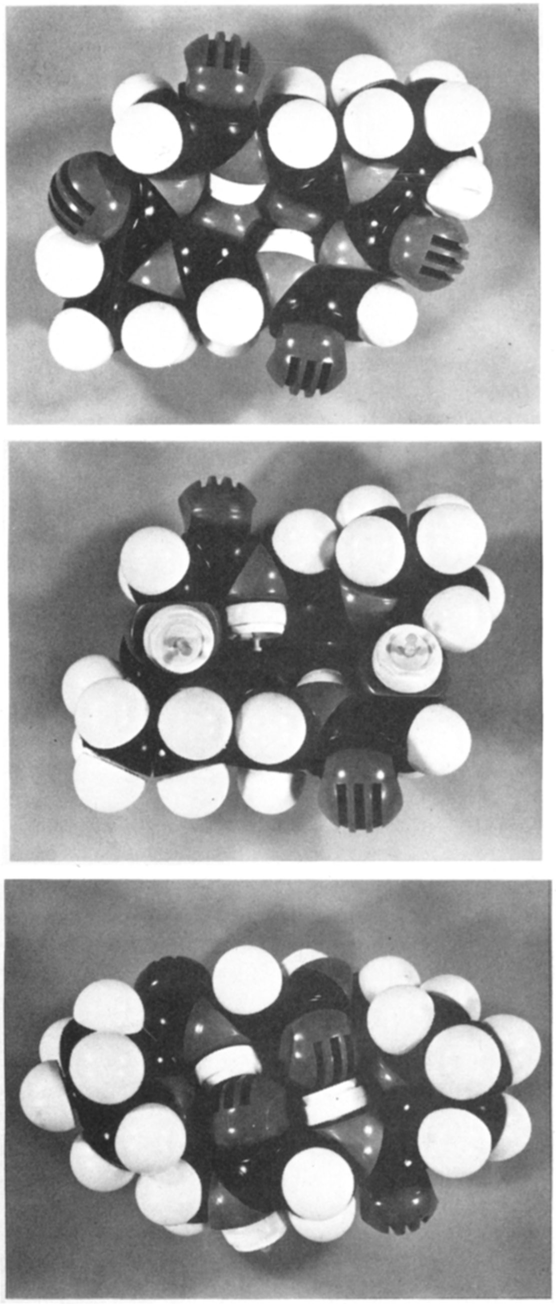


FIGURE 3: Photographs of Corey–Pauling–Koltun models of proposed conformations of *cyclo*-(X-L-Pro-Y)$_2$ peptides: (a, top) conformer with type II β turns and γ turns; (b, middle) conformer with type I β turns; (c, bottom) conformer with type II′ β turns. *cyclo*-(Gly-L-Pro-Gly)$_2$ is shown in all three cases for clarity of viewing the peptide backbone.

As shown by the data in Table V, the results for *cyclo*-(Gly-L-Pro-D-Y)$_2$ peptides are closely analogous to those for peptides with L residues preceding the Pro's, but the γ turn contribution seems reduced (larger value of $\Delta\delta_{\beta\gamma}$), and the propensity toward populating a two-cis conformation is absent. Concurrently, the chemical shift of the solvent-shielded NH's is at higher field, signaling a lessened involvement in intramolecular hydrogen bonding. These differences can be accounted for by an altered β conformation (see Table VI) or alternatively by an equilibrium (rapidly established on the NMR time scale) between two conformations, one of which contains no γ turn contribution. Since the temperature-dependence data do not reflect a sharp distinction in accessibility between the X and Y N–H's, dynamic averaging is a viable possibility.[3] Results for *cyclo*-(Gly-L-Pro-Gly)$_2$ are even more suggestive of conformational averaging, since its CD spectra are broad and have smaller ellipticities than those of the other peptides.

In the cases of the *cyclo*-(D-X-L-Pro-D-Y)$_2$ and *cyclo*-(Gly-L-Pro-D-Y)$_2$ peptides examined, the data again support a β conformation with the X residues extended. Here the observation of type C′ CD spectra (lacking a long-wavelength minimum) allows the conclusion to be drawn that the Pro ψ angles are trans′ and that no strong γ-turn contribution is present (also consistent with the larger $\Delta\delta_{\beta\gamma}$). Dihedral angles of an approximate conformation satisfying these requirements and consistent with coupling constants are given in Table VI.

The introduction of L residues following the prolines in the cyclic hexapeptides causes pronounced changes in spectral parameters which can be correlated with a preference for conformers containing cis′ proline ψ angles. Whether the X or Y residues are extended (i.e., show solvent shielding of their N–H's) depends upon the nature of L-Y and upon the presence of Gly's or D residues as X. *cyclo*-(Gly-L-Pro-L-Ala)$_2$, *cyclo*-(Gly-L-Pro-L-Phe)$_2$, and *cyclo*-(Gly-L-Pro-L-Ser)$_2$ (Torchia et al., 1972a) all prefer β conformations with intramolecular hydrogen bonding of the X residues (as indicated by temperature and solvent dependences of N–H resonances) and with type I -Pro-L-Y- β turns. Evidence for the latter conclusion is principally the observation of type C CD spectra (for *cyclo*-(Gly-L-Pro-L-Ala)$_2$ which were found among calculated spectra when the Pro ψ angles were cis′.[4] Dihedral angles for the proposed β conformation containing type I β turns are given in Table VI, and a photograph of a CPK model is shown in Figure 3b.

Both *cyclo*-(Gly-L-Pro-L-Val)$_2$ and *cyclo*-(D-Phe-L-Pro-L-Val)$_2$ take up all-trans conformations in which the L-Val N–H's are less accessible to solvent than the X residue N–H's, as would be expected for a β structure with type II′ D-Phe- or Gly-L-Pro β turns. Again, the Pro ψ angles would be proposed to be cis′ (as indicated by the CD data). This conformation (see Table VI and Figure 3c) is equivalent to that proposed by Kopple et al. (1973) for *cyclo*-(D-Phe-L-Pro-L-Ala)$_2$, *cyclo*-(D-Phe-L-Pro-L-Orn)$_2$, and *cyclo*-(D-Phe-L-Pro-L-His)$_2$.

The *cyclo*-(Gly-L-Pro-L-Y)$_2$ peptides (but not those with D-X residues) adopted to varying extents an asymmetric conformation with one-cis peptide bond, as had been previously described for *cyclo*-(Gly-L-Pro-L-Ser)$_2$ (Torchia et al., 1972a). Unlike the other conformational equilibrium seen between all-trans and two-cis structures, here no clear correlation was evident between side-chain character or solvent polarity and the all-trans/one-cis distribution (Table III). Spectral complexity precluded detailed conformational analysis of the one-cis structure.

Discussion

Preferred β Structures. A set of "rules" correlating amino acid sequence with preferences for particular types of β-turn structures in the cyclic hexapeptides can be formulated on the basis of results discussed above.

(I) -L-Pro-Gly- or -L-Pro-D-Y sequence → type II β turns (trans′ $i + 1$ Pro)

(II) -L-Pro-L-Y
  (A) -Gly-L-Pro-L-Y (Y not L-Val) → type I β turns (cis′ $i + 1$ Pro)
  (B) -Gly-L-Pro-L-Val- or -D-X-L-Pro-L-Y- → type II′ β turns (cis′ $i + 2$ Pro's)

These "rules" are consistent with and reinforce Venkatachalam's (1968) original stereochemical predictions [as subsequently refined by Chandrasekaran et al. (1973)]. Moreover, they extend the correlation of sequence to preferred turn type by implicating two specific factors, previously not identified, as conformational determinants: (1) the steric bulk of an L residue following L-proline (a β-branched residue, L-Val, makes type I turns less likely, and favors II′); and (2) the nature of the residue preceding L-proline (a D residue makes type II′ turns more likely than would a glycine; an L residue leads to a tendency toward γ-turn hydrogen bonding in conjunction with the β turn).

Note that there are potential limits to the application of the above rules. In cyclic hexapeptides, no predictions can be made for the case when both X and Y are L residues; no such examples have been analyzed because these materials cannot be readily synthesized. It is likely, given the available results, that cis conformers would occur. Furthermore, the extent to which results obtained on cyclic hexapeptide systems are generalizable to other peptides, particularly linear ones, or proteins, remains to be demonstrated.

Comparisons with Crystal Structures. A number of crystal structures of *cyclo*-(X-L-Pro-Y)$_2$ peptides are available (Brown & Teller, 1976; Kostansek et al., 1979a,b; Brown & Yang, 1979; Flippen-Anderson & Karle, 1979). For purposes of comparison, dihedral angles of the various peptides in crystals are given in Table VIIA. Peptides with several different types of sequence are represented.

Although strict C_2 symmetry is observed in the crystal conformation of only *cyclo*-(L-Ala-L-Pro-D-Phe)$_2$, deviations from a 2-fold rotational symmetry in individual values of ϕ, ψ, ω are quite small in all cases (*except* one critical part of the *cyclo*-(Gly-L-Pro-Gly)$_2$ structure (vide infra)). This finding of real or approximate C_2 symmetry in dihedral angles of these peptides is interesting since the NMR experiments reveal only a dynamically averaged structure and do not indicate whether the actual situation is one of rapid fluctuations between two or more conformations. No direct comparison can be made, of course, between observations in solution and in crystals, since intermolecular interactions may be highly contributory in the latter. Nonetheless, the structures seen in the solid state reveal possible relatively low-energy conformers for the peptides.

The data in Table VIIB provide insight into potential intramolecular hydrogen bonding in the *cyclo*-(X-L-Pro-Y)$_2$ peptides. In only two cases are "strong" hydrogen bonds seen [*cyclo*-(Gly-L-Pro-D-Ala)$_2$, N···O = 3.04 Å; *cyclo*-(Gly-L-Pro-Gly)$_2$, N···O = 2.91 Å], and in each of these examples, there is only *one* good intramolecular hydrogen-bonding interaction. Madison (1974) has pointed out that calculated energies for the cyclic hexapeptides show a destabilizing electrostatic contribution when two "good" (N···O = 2.8–3.0 Å) intramolecular hydrogen bonds are present. The repulsive component arises from a short transannular O···O contact (2.3 to 2.5 Å). He suggested that what has now been seen in the crystal structures, i.e., formation of *one* hydrogen bond, could

---

[3] A cyclic hexapeptide system which appears to undergo "frame-shift" averaging has been reported by Kopple et al. (1978): the *cyclo*-(Gly-D-X-L-Y)$_2$ compounds, where neither X nor Y is Pro.

[4] In a recent discussion of CD data for *cyclo*-(X-L-Pro-Y)$_2$ peptides (Bush et al., 1978), type C spectra were correlated with 2′ turns. ^{1}H NMR data on *cyclo*-(Gly-L-Pro-L-Ala)$_2$, and the related compounds *cyclo*-(Gly-L-Pro-L-Phe)$_2$ and *cyclo*-(Gly-L-Pro-L-Ser)$_2$, clearly support the conclusion that these compounds adopt a conformation with the Gly NH's intramolecularly hydrogen bonded. This finding and others led to assigning type 1 turns to these β conformers (not 2′). Inspection of calculated spectra leads to the more general correlation [than that of Bush et al. (1978)] of type C spectra with cis′ Pro species (both types 1 and 2′ turns).

Table VII

(A) Dihedral Angles (Degrees) of *cyclo*-(X-L-Pro-Y)$_2$ Peptides in Crystals

	Y			L-Pro			Y			
peptide	ϕ	ψ	ω	ϕ	ψ	ω	ϕ	ψ	ω	ref
cyclo-(L-Ala-L-Pro-D-Phe)$_2$	−157	172	178	−60	122	171	78	9	−169	Brown & Teller (1976)
cyclo-(Gly-L-Pro-D-Ala)$_2$	−179	170	−175	−54	125	174	94	−5	−174	Kostansek et al. (1979a)
	−173	−163	−173	−70	116	174	79	19	176	
cyclo-(Gly-L-Pro-D-Phe)$_2$	178	164	170	−56	134	167	100	−9	173	Brown (1979)
	165	162	177	−72	131	174	112	−19	169	
cyclo-(Gly-L-Pro-Gly)$_2$	−142	−173	−177	−66	−36	−173	−115	−7	−177	Kostansek et al. (1979b)
	−150	178	180	−53	126	179	83	−3	−175	
cyclo-(D-Phe-L-Pro-L-Val)$_2$	60	−134	−162	−86	−15	−177	−136	155	−176	Flippen-Anderson & Karle (1979)
	52	−138	−171	−97	4	−172	−137	124	180	

(B) Transannular N· · ·O and O· · ·O Distances (Å) in *cyclo*-(X-L-Pro-Y)$_2$ Peptides in Crystals[a]

peptide	N· · ·O	O· · ·O	peptide	N· · ·O	O· · ·O
cyclo-(L-Ala-L-Pro-D-Phe)$_2$	3.20	2.90	*cyclo*-(Gly-L-Pro-Gly)$_2$	2.91,[b] 3.95[c]	3.04
cyclo-(Gly-L-Pro-D-Ala)$_2$	3.04, 3.42	2.83	*cyclo*-(D-Phe-L-Pro-L-Val)$_2$	3.40, 3.77	
cyclo-(Gly-L-Pro-D-Phe)$_2$	3.49, 3.56	2.84			

[a] See references above. [b] Type II β turn. [c] Type I β turn.

alleviate the unfavorable contacts. It is possible that in solution a rapid making/breaking of the hydrogen bonds occurs. Otherwise, it appears that little stabilization of the conformation would be provided by the intramolecular hydrogen bonds.

The general types of β conformers seen in the crystal structures are the same as those proposed in solution, with the notable exception of *cyclo*-(Gly-L-Pro-Gly)$_2$, where one half of the molecule adopts a type II β turn (as proposed in solution) and the other half a type I. No conclusions are possible with respect to the likelihood of a dynamic average between these two types of turn in solution, although solution data do suggest that averaging occurs (vide supra). No suggestion of a γ-turn contribution to the conformations is found in the X-ray structures.

Implications for Preferred Turns in Natural Peptides and Proteins. Even though certain aspects of the cyclic hexapeptide results are specific to these peptides (e.g., the transannular O···O repulsive contact; vide supra), much of what has been observed can be related to natural peptides and to proteins.

Gramicidin S is an antibiotic whose activity has been postulated to depend on proximity of this decapeptide's two ornithine side chains (Ovchinnikov & Ivanov, 1975; Ivanov, 1977). The maintenance of the requisite side-chain arrangement is apparently accomplished by the rigid conformation containing two -D-Phe-L-Pro- type II′ β turns. Results presented here indicate that a -D-X-L-Pro- sequence is not a sufficient condition for II′ turn formation. However, consistent with the hexapeptide findings, the gramicidin S sequence has L-valines following the L-prolines—which would ensure a preference for II′ turns (as in *cyclo*-(D-Phe-L-Pro-L-Val)$_2$).

A survey of β turns in a group of proteins (Chou & Fasman, 1977; Smith & Pease, 1980) revealed statistics for frequencies of amino acid occurrence in β turns which are entirely consistent with the hexapeptide derived "rules". In the sample of 210 β turns (Smith & Pease, 1980), only 16 examples of II′ turns were seen. This is not surprising given the absence of D residues in proteins. Proline occurred 31 times overall, yet only once in position $i + 2$. Of its 31 occurrences, 19 were in the $(i + 1)$th position of type I turns. Glycine was the most frequent residue overall, with 61 occurrences. Of these, 14 were in position $i + 1$ of type II turns while another 23 were in the $i + 2$ position of type II turns. Valine, which was not a frequent amino acid in the turns, was found 8 times out of its 12 occurrences in position $i + 1$ of type I or III (a 3_{10} helical turn, close in ϕ, ψ to type I) nonproline-containing turns.

Two further observations from this study of cyclic hexapeptides which may offer insight into β turns in proteins are (1) the reduced contribution of the $i + 3 \rightarrow i$ hydrogen bonding to type I and II turns relative to type II turns (judged by N–H chemical shifts), and (2) the likelihood of simultaneous $i + 2 \rightarrow i$ and $i + 3 \rightarrow i$ hydrogen bonding.

cyclo-(X-L-Pro-Y)$_2$ peptides thus provide a model system for β turns, where results with peptides of varying X and Y residues have provided a set of "rules" for correlating amino acid sequence with preferred β conformers. These rules are potentially useful in gaining insight into conformational states of natural peptides and proteins.

Acknowledgments

We are grateful to Drs. Paul Young and Kenneth Kopple for making available cyclic peptide samples and for stimulating discussions. We also thank Dr. Eric Fossel for running several of the NMR spectra. Technical assistance by David A. Gilbert, Brian M. Bingaman, and Steven C. Freilich is gratefully acknowledged.

References

Brown, J. N., & Teller, R. G. (1976) *J. Am. Chem. Soc. 98*, 7565.

Brown, J. N., & Yang, C. H. (1979) *J. Am. Chem. Soc. 101*, 445.

Bush, C. A., Sarkar, S. K., & Kopple, K. D. (1978) *Biochemistry 17*, 4951.

Bystrov, V. F., Portnova, S. L., Balashova, T. A., Kozmin, S. A., Gavrilov, Yu. D., & Afanas'ev, Ya. (1973) *Pure Appl. Chem. 36*, 19.

Chandrasekaran, R., Lakshminarayan, A. V., Pandya, U. V., & Ramachandran, G. N. (1973) *Biochim. Biophys. Acta 303*, 14.

Chou, P. Y., & Fasman, G. D. (1977) *J. Mol. Biol. 115*, 135.

Deber, C. M., & Blout, E. R. (1974) *Isr. J. Chem. 12*, 15.

Deber, C. M., Madison, V., & Blout, E. R. (1976) *Acc. Chem. Res. 9*, 106.

DeMarco, A., Llinás, M., & Wüthrich, K. (1978) *Biopolymers 17*, 637.

Easwaran, K. R. K., Pease, L. G., & Blout, E. R. (1979) *Biochemistry 18*, 61.

Flippen-Anderson, J. L., & Karle, I. L. (1979) *Proc. Am. Pept. Symp. 6th*, 145.

Grathwohl, Ch., Schwyzer, R., Tun-Kyi, A., & Wüthrich, K. (1973) *FEBS Lett. 29*, 271.

IUPAC–IUB Commission on Biochemical Nomenclature (1970) *Biochemistry 9*, 3471.

Ivanov, V. T. (1977) *Proc. Am. Pept. Symp., 5th*, 307.

Karle, I. L. (1974) *J. Am. Chem. Soc. 96*, 4000.

Karle, I. L., Karle, J., Wieland, T., Burgermeister, W., Faulstich, H., & Witkop, B. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 1836.

Karle, I. L., Karle, J., Wieland, T., Burgermeister, W., & Witkop, B. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1782.

Kopple, K. D., Ohnishi, M., & Go, A., (1969a) *J. Am. Chem. Soc. 91*, 4264.

Kopple, K. D., Ohnishi, M., & Go, A. (1969b) *Biochemistry 8*, 4087.

Kopple, K. D., Go, A., Logan, R. H., Jr., & Savdra, J. (1972) *J. Am. Chem. Soc. 94*, 973.

Kopple, K. D., Go, A., Schamper, T. J., & Wilcox, C. S. (1973) *J. Am. Chem. Soc. 95*, 6090.

Kopple, K. D., Go, A., & Schamper, T. J. (1978) *J. Am. Chem. Soc. 100*, 4289.

Kostansek, E. C., Lipscomb, W. N., & Thiessen, W. E. (1979a) *J. Am. Chem. Soc. 101*, 834.

Kostansek, E. C., Thiessen, W. E., Schomburg, D., & Lipscomb, W. N. (1979b) *J. Am. Chem. Soc. 101*, 5811.

Laiken, S., Printz, M., & Craig, L. C. (1969) *J. Biol. Chem. 244*, 4454.

Madison, V. (1973) *Biopolymers 12*, 1837.

Madison, V. (1974) in *Peptides, Polypeptides and Proteins* (Blout, E. R., Bovey, F. A., Goodman, M., & Lotan, N., Eds.) pp 89–98, Wiley, New York.

Madison, V., & Kopple, K. D. (1980) *J. Am. Chem. Soc. 102*, 4855.

Madison, V., Atreyi, M., Deber, C. M., & Blout, E. R. (1974) *J. Am. Chem. Soc. 96*, 6725.

Ovchinnikov, Yu. A., & Ivanov, V. T. (1975) *Tetrahedron 31*, 2177.

Ovchinnikov, Y. A., Ivanov, V. T., Bystrov, V. F., Miroshnikov, A. I., Shepel, E. N., Abdullaev, N. D., Efremov, E. S., & Senyavina, L. B. (1970) *Biochem. Biophys. Res. Commun. 39*, 217.

Patel, D. J. (1973a) *Biochemistry 12*, 496.

Patel, D. J. (1973b) *Biochemistry 12*, 677.

Patel, D. J., & Tonelli, A. E. (1973) *Biochemistry 12*, 486.

Pease, L. G. (1975) Ph.D. Dissertation, Harvard University, Cambridge, MA, University Microfilms, Ann Arbor, MI, No. 75–23, 169.

Pease, L. G., & Watson, C. (1978) *J. Am. Chem. Soc. 100*, 1279.

Pease, L. G., Deber, C. M., & Blout, E. R. (1973) *J. Am. Chem. Soc. 95*, 258.

Pease, L. G., Niu, C.-H., & Zimmermann, G. (1979) *J. Am. Chem. Soc. 101*, 184.

Portnova, S. L., Shilin, V. V., Balashova, T. A., Biernat, J., Bystrov, V. F., Ivanov, V. T., & Ovchinnikov, Yu. A. (1971) *Tetrahedron Lett. 33*, 3085.

Ramachandran, G. N., Chandrasekaran, R., & Kopple, K. D. (1971) *Biopolymers 10*, 2113.

Reed, L. L., & Johnson, P. L. (1973) *J. Am. Chem. Soc. 95*, 7523.

Schwyzer, R., Grathwohl, Ch., Meraldi, J.-P., Tun-Kyi, A., Vogel, R., & Wüthrich, K. (1972) *Helv. Chim. Acta 55*, 2545.

Siemion, I. Z., Wieland, T., & Pook, K. H. (1975) *Angew. Chem., Int. Ed. Engl. 14*, 702.

Smith, J. A., & Pease, L. G. (1980) *CRC Crit. Rev. Biochem. 8*, 315.

Sogn, J. A., Craig, L. C., & Gibbons, W. A. (1974) *J. Am. Chem. Soc. 96*, 3306.

Torchia, D. A., di Corato, A., Wong, S. C. K., Deber, C. M., & Blout, E. R. (1972a) *J. Am. Chem. Soc. 94*, 609.

Torchia, D. A., Wong, S. C. K., Deber, C. M., & Blout, E. R. (1972b) *J. Am. Chem. Soc. 94*, 616.

Urry, D. W., & Walter, R. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 956.

Urry, D. W., Ruiter, A. L., Starcher, B. C., & Hinners, T. A. (1968) *Antimicrob. Agents Chemother.*, 87.

Venkatachalam, C. M. (1968) *Biopolymers 6*, 1425.

Woody, R. W. (1974) in *Peptides, Polypeptides and Proteins* (Blout, E. R., Bovey, F. A., Goodman, M., & Lotan, N., Eds.) pp 338–350, Wiley, New York.